PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference WPP89320	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2005/003043	International filing date *(day/month/year)* 11.03.2005	Priority date *(day/month/year)* 11.03.2004

International Patent Classification (IPC) or national classification and IPC
INV. C07K14/16 C07K16/10 A61K39/21 A61K39/42

Applicant
ISTITUTO SUPEIORE DI SANITA et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11.01.2006	22.06.2006

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2005/003043

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-26 as originally filed

   Sequence listings part of the description, Pages

   1-6 as originally filed

   Claims, Numbers

   1-33 received on 20.04.2006 with letter of 20.04.2006

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 34
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 1, 2, 4
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 19-28, 32

because:

☒ the said international application, or the said claims Nos. 19-28, 32 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*.

☐ no international search report has been established for the said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8, 10, 11, 13-22, 24-34
	No:	Claims	1-7, 9, 12, 23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	8, 10, 11, 13-22, 24-34
Industrial applicability (IA)	Yes:	Claims	1-18, 30, 31, 33
	No:	Claims	19-28, 31 (opinion reserved)

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

1. Re Item I
Basis of the opinion

Claims 1 and 2 have been amended to specify "wherein the V3 loop is coordinated with a binding region". No basis for this amendment was provided. The term "coordinated" does not appear to be used anywhere in the description. In the absence of a basis in the application as originally filed, this amendment cannot be accepted.

Claim 4 has been amended to refer to Tat as "non-oxidised"; however, no basis in the application for this term was given. Tat is referred to in the description as "native", but not the more specific "non-oxidised". Thus, Claim 4 as amended is not considered to have a basis in the application as originally filed.

In the absence of suitable basis, Claims 1, 2 and 4 are considered as if the amendments had not been made, and the claims are considered to be as originally filed.

2. Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 19-28 and 31 are considered to encompass subject-matter which is considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

3. Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

3.1 Novelty

3.1.1 Reference is made to the following documents:

D1: WO 01/54719

D2: Lee B et al (1999), J. Biol. Chem., vol. 274, pages 9617-9626
D3: Gzyl J et al (2004), Virology, vol. 318, pages 493-506
D4: Wyatt R et al (1995), J. Virol., vol. 69, pages 5723-5733

D3 and **D4** are newly cited; a copy of each is enclosed.

3.1.2 **D1** discloses the use of an HIV Tat protein and an HIV gp120 protein in the manufacture of a vaccine for immunisation against HIV (abstract).

On page 4 of the present application, it is said that in the preparation of **D1**, Tat is not able to bind the V3 loop of Env (gp120) since Env has not been activated by CD4.

The applicant argued on one occasion that the V3 loop is only exposed in the following conditions: full length Env activated by CD4 or heparin sulphates, isolated V3, or V2 deletions forms of gp120, gp140, gp160 and Env. Thus the applicant considers that full length Env alone does not expose the V3 loop, and thus cannot form the claimed complex. However, the application says that the combination of Tat with **wild type Env** results in the formation of a new molecular species (page 22). Page 23 states that Tat/Env complexes are novel immunogens. This teaching is not consistent with the applicant's arguments.

On a subsequent occasion the applicant argued that the V3 loop can be made available by various means including trimerisation. However, the applicant did not indicate where the description covers this aspect, and the term "trimerisation" does not appear to be used anywhere in the description.

Claim 1 of the present application specifies that the V3 loop of gp120 is available to coordinate with a binding region of SEQ ID NO.1 (Tat). If wild-type Env must be modified in order to expose the V3 loop, then Claim 1 lacks an essential feature by which the "available to coordinate" effect is achieved. The structural means by which the loop is "made available" is an essential feature, required to distinguish the subject-matter from **D1**. In its absence, Claim 1 cannot be distinguished from **D1**, especially in light of the teaching on pages 22 and 23, which imply that wild-type Env can form the desired complex with Tat.

Thus, **D1** is novelty destroying for Claims 1-6, 8 and 11.

3.1.3 Since the terms "complex" and "coordinate" also appear to be necessary to distinguish the entity of Claim 1 from that of **D1**, they should be completely clear and unambiguous. This is not the case. The application (page 5) describes a complex as a "combination", or the two peptide species "in contact with each other", and says that the complex may relay simply on the "natural interaction between Tat and the V3 loop of gp120", but that "weaker complexes may also be employed". The complex "may simply comprise the relevant areas of Tat and gp120". However, the entire proteins of Tat and gp120 may also be used (page 6 "the peptide comprising the V3 loop may comprise or consist of gp120" and page 9 "it is generally preferred that...substantially the full sequence of Tat").

The applicant has tried to distinguish the Tat-Env composition of **D1** from the complex of Claim 1 by arguing that in **D1** "Tat is simply combined but not bound to gp120". However, page 5 describes the complex as a "combination", which suggests that the components are combined, as in **D1**.

3.1.4 Claims 16 and 17 specify where the Tat binding region is located within Tat; this does not however, restrict the size of the Tat fragment included in the complex.

Since this does not seem to change the component parts of the complex of Claim 1, it is considered that these features also lack novelty over **D1**.

3.1.5 Claim 22 is directed to an antibody defined by reference to its production process. D2 describes antibodies to CCR5 second extracellular loop, which apparently also bind Tat. Since a product is not made novel by means of its production method, the antibodies of **D2** are novelty-destroying for Claim 22.

The applicant's attention is brought to the fact that Claim 22 does not define the antibody in terms of its binding characteristics. Thus, even if an antibody is raised to a complex, it may only recognise one of the complex components.

Furthermore, it is noted that where the application relates to antibody production, it is mentioned that antibodies raised to the complex bound HIV gp41 (page 24). This would seem that antibodies to gp41 would be novelty destroying for Claim 22.

3.2 Inventive step

3.2.1 In addition to the relevance of **D1** alone with respect to novelty, the following comments regarding its relevance for inventive step should be considered.

D3 discloses attempts to increase the immunogenicity of the Env peptide. One attempt which involves the deletion of the V1 and V2 variable domains and modification of the V3 loop (ΔV1/V2/mV3) produced some of the highest level of cross-reactive responses (page 497).

D4 discloses involvement of the V1/V2 variable loop structure in the exposure of gp120 epitopes induced by CD4 binding. D4 considers that the V2 loop is especially involved in partially masking epitopes on the native gp120 monomer.

D1 is considered the closest prior art. The difference between the composition of **D1** and that of Claim 1 is that in Claim 1 the V3 loop of Env is exposed, leading to better vaccine activity. The problem to be solved may be formulated as provision of an improved HIV vaccine. The skilled person, starting from **D1**, and looking to improve the Env-Tat vaccine, would be aware of the teaching of **D3** which directs the removal of the V1 and V2 variable domains of the Env protein. Similar teaching is also present in **D4**, where the V1 and V2 loops are said to mask epitopes in the absence of CD4.

Thus, the skilled person would be motivated to replace the wild-type Env of **D1** with the deletion mutant of either one of **D3** or **D4**, resulting in an Env in which the V3 loop is better exposed for binding to Tat, and the subject-matter of Claim 1 lacks an inventive step.

3.2.2 Claims 9 and 10 specify that the gp160 lacks the V2 loop of gp120. Claim 18 specifies that the complex peptides are cross-linked.

These features are not considered to confer any surprising technical effect on the complex of Claim 1, and thus are not suitable for conferring an inventive step on the claims. It is also noted that the application does not appear to list any suitable cross-linking reagents.

3.2.3 Claim 12 relates to a further components of the complex, which component is

capable of interacting with Env to expose a functional V3 loop. Claim 13 specifies that the component is CD4, or a fragment thereof.

The description does not disclose how CD4 should be added to the complex; the application merely states that the "exposure [of the V3 loop] may be achieved by adding CD4, or the gp120 binding epitope of CD4" (page 9). The gp120 binding epitope of CD4 is not disclosed, nor are fragments or variants of CD4 which bind gp120 and induce the required conformational changes. In fact, no complex comprising CD4 is actually disclosed in the application, it would appear that none was ever used.

3.2.4 Claims 14 and 15 relate to further components of the complex of Claim 1.

The description (page 8) does not disclose any surprising technical effect associated with the presence of the further components, and as such, they appear to be mere obvious additions proposed on the basis of their known role in Env binding to cells, and not in themselves inventive.

3.2.5 The features of Claims 19-33 are trivial variants which are not suitable for conferring an inventive step on the independent claims to which they ultimately refer.

3.3 Industrial applicability

Claims 19-28 and 31 are directed to subject-matter which is considered to encompass a **method of treatment or surgery of the human or animal body**.

For the assessment of these claims on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims (Rule 39 PCT). The EPO, for example, does not recognise as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

4. Re Item VIII

Certain observations on the international application

4.1 The claims as a whole lack clarity and conciseness (Article 6 PCT) due to the presence of more than one independent claim directed to the same subject-matter. For example, independent Claims 1 and 2 are both directed to a complex comprising first and second peptides.

4.2 Claim 3 refers to an entity by means of citing a journal article. Such an incorporation of information into a claim by reference to a document is very uncommon and renders the claim prima facie unclear. In any event, it appears that the cited article relates to more than one monoclonal antibody directed against the CCR5 second extracellular loop, and **it cannot be determined exactly which antibody is intended**. The applicant's attention is brought to the fact that EPO case law is not relevant during the PCT phase, and in any event, need not be followed during a regional phase.

4.3 It is observed that the nomenclature throughout the description lacks consistency; gp120 is often referred to as Env (in the prior art, Env is also called gp160 and is a precursor of gp120. Env may also refer to a complex between gp120 and gp41; see page 1 of the application), creating uncertainty as to the sequence intended (for example, see the first two paragraphs of page 4).

4.4 The claims are cast using language that is highly functional in nature, without reference to technical features which enable the functional features to be obtained. This omission of essential technical features renders the claims unclear.

Examples of such functional language:
Claim 1 "the V3 loop being available", and "fragment, mutant or variant thereof capable of binding"; Claim 3 "being recognisable by the monoclonal antibody"; Claim 4 "biologically active Tat"; Claims 6 and 8 "capable of binding a peptide"; Claim 12 "capable of interacting with Env"; Claim 14 "capable of binding said heparan sulphate"; Claim 16 "generatable by proteasomes".

4.5 Claim 23 relates to an antibody "obtained in accordance with any of Claims 20 to 22. However, Claims 20 to 22 are use claims, not method or process claims.

4.6 The term "combination" used in Claim 29 lacks clarity, since the term is open to interpretation. Furthermore, it is not certain whether the claim relates to a complex, or to a combination.

4.7 The applicant's attention is brought to the fact that the application includes references to documents which were not publicly available at the filing date. For example, see reference to PCT/EP2004/11950 on pages 7, 12, 25 and 26, and reference to "Rezza et al, J. Infect. Dis, in press" on page 22. Such references cannot be used as disclosure.

Claims

1. A complex comprising first and second peptides, the first peptide comprising the V3 loop of gp120, and wherein the V3 loop is coordinated with a binding region on the second peptide, the binding region comprising at least residues 21-40 and 46-58 of SEQ ID NO 1, or a fragment, mutant or variant thereof capable of binding residues 301-419 of SEQ ID NO. 2.

2. A complex comprising first and second peptides, the first peptide comprising the V3 loop of gp120, and wherein the V3 loop is coordinated with a binding region on the second peptide, the binding region being derived from Tat and being recognisable by the monoclonal antibody directed against the CCR5 second extracellular loop described by Lee, B., *et al.*, J. Biol. Chem., 1999, Vol. 274, 9617-9626.

3. A complex according to claim 1 or 2, wherein the binding region comprises at least residues 21-58 of SEQ ID NO 1, or a fragment, mutant or variant thereof capable of binding residues 301-419 of SEQ ID NO. 2.

4. A complex according to any preceding claim, prepared with non-oxidised Tat.

5. A complex according to any preceding claim, wherein the peptide comprising the V3 loop comprises some or all of Env in addition to the V3 loop.

6. A complex according to any preceding claim, wherein the peptide comprising the V3 loop comprises the complete sequence of SEQ ID NO 2, or a fragment, variant or mutant thereof capable of binding a peptide consisting of residues 21-58 of SEQ ID NO 1.

7. A complex according to any preceding claim, wherein the peptide comprising the V3 loop consists of the V3 loop region of gp120.

8. A complex according to any preceding claim, wherein the peptide comprising the V3 loop comprises at least residues 301-419 of SEQ ID NO. 2, or a fragment, variant or mutant thereof capable of binding a peptide consisting of residues 21-58 of SEQ ID NO 1.

9. A complex according to any preceding claim, having all or part of gp160 as a component thereof, the gp160 comprising at least the V3 loop of gp120 and lacking at least the majority of the V2 loop of gp120.

10. A complex according to any preceding claim, having ΔV2Env as a component thereof.

11. A complex according to any preceding claim, wherein the peptide comprising the V3 loop comprises at least residues 301 to 419 as shown in SEQ ID NO. 2.

12. A complex according to any preceding claim, further comprising a molecule or substance capable of interacting with Env to expose a functional V3 loop.

13. A complex according to claim 12, wherein said molecule or substance is CD4 or a fragment, mutant or variant thereof.

14. A complex according to any preceding claim, further comprising a heparan sulphate, optionally further comprising at least one other molecule capable of binding said heparan sulphate.

15. A complex according to any preceding claim, further comprising a substance selected from integrins, basic fibroblast growth factor, CD26, VEGF receptors, and chemokine receptors.

16. A complex according to any preceding claim, wherein the binding region is contained within a fragment of Tat generatable by proteasomes of human cells on exposure to Tat.

17. A complex according to claim 16, wherein the Tat fragment is selected from: fragments containing the cysteine, basic and RGD regions of Tat; fragments containing the cysteine and basic regions of Tat; fragments containing the basic and RGD region of Tat; and, fragments containing the basic region of Tat, alone.

18. A complex according to any preceding claim, wherein said peptides are cross-linked.

19. Use of a complex according to any preceding claim to generate antibodies thereagainst.

20. Use according to claim 19 in a process to obtain a monoclonal cell line.

21. Use according to claim 19 or 20, wherein the antibodies are selected such as not to recognise any of the epitopes of the group of native Tat, gp160, CD4 or gp120, CCR5, and the V3 loop region of gp120 also recognized by antibodies generated by one of the group when used as immunogen in isolation but only as a complex according to any of claims 1 to 19.

22. An antibody obtained by a process as defined in any of claims 19 to 21.

23. The antibody of claim 22 which is humanised to prevent or reduce an adverse immune reaction on injection into a human.

24. Use of the antibody of claim 223 or 23 in prophylactic or therapeutic passive immunisation against a virus infection, wherein said virus expresses Tat.

25. Use according to claim 24, wherein said virus is HIV.

26. Use of claim 24 or 25, wherein the recipient is an expectant or nursing mother.

27. Use of a complex according to any of claims 1 to 18 as an immunogen for vaccination.

28. Use according to claim 27, wherein said virus is HIV.

29. A complex according to any of claims 1 to 18, provided as a combination of the peptides in a vehicle suitable for injection.

30. A kit comprising at least two separate preparations of the components of a complex according to any of claims 1 to 18.

31. Use of a complex according to any of claims 1 to 18 in therapy.

32. Use of a complex according to any of claims 1 to 18, in the preparation of a medicament for the treatment or prophylaxis of a viral infection, whereby the infecting

virus expresses a molecule capable of forming a ternary complex between said molecule, CD4 and CCR5.

33. Use of a complex according to any of claims 1 to 18 to establish whether a sample from a patient contains antibodies against said complex.